Biochemistry

 Volume 32, Number 10 March 16, 1993

Accelerated Publications

Myosin-ATP Chemomechanics†

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Received December 11, 1992; Revised Manuscript Received January 19, 1993

ABSTRACT: The hydrodynamic size of rabbit skeletal muscle myosin subfragment 1 (S1) is decreased when S1 and MgATP form the steady-state intermediate S1-MgADP,P_i. The rotational decay time, τ, determined by transient electrical birefringence techniques was 259 ns for S1-MgADP,P_i and 271 ns for S1-MgADP at 3 °C in low ionic strength solutions. The data were interpretated using a hydrodynamic model consisting of a rigid linear four-bead structure that had a point at the center of one of the inner beads about which the structure can bend. The structure of S1-MgADP was approximated by adjusting the bend angle to 20°. The best fit to the S1-MgADP,P_i decay time was then obtained when the angle was increased to 38°. The results obtained using this simple model suggest that MgATP binding and hydrolysis changes the structure of S1 so that one end of it moves by at least 3.9 nm. The reverse of this process, during product release, would provide a displacement large enough to account for most of the ATP-driven filament sliding that occurs in muscle or in in vitro motility assays.

The subfragment 1 (S1)[1] portion of the myosin cross-bridge binds MgATP and actin, and the acto-S1-MgATP complex generates force. There are, a priori, two broad categories of structural change that S1 can contribute in this process. One is the reorientation relative to the actin filament axis of a structurally unchanged S1. The other is a change in the shape of S1 itself with little or no change in its orientation at the actin interface. Mechanical (Huxley & Simmons, 1973), extrinsic probe orientation (Borejdo et al., 1982; Cooke et al., 1982; Ajtai & Burghardt, 1989; Tanner et al., 1992), and X-ray diffraction (Matsuda & Podolosky, 1984; Harford & Squire, 1992) measurements suggest that at least a portion of the actin-bound cross-bridge moves during force production, but the relative contributions of reorientation and/or shape change are not known.

We have observed an ATP-induced change in S1 structure by measuring the rates of S1-nucleotide rotational Brownian motion in solution using the technique of transient electric birefringence. This technique does not involve extrinsic probes and is very sensitive to S1 structural changes (Highsmith & Eden, 1986).

MATERIALS AND METHODS

S1 with only alkali light chain 1 present was isolated from rabbit skeletal muscle and purified by ion exchange chromatography as described (Weeds & Taylor, 1976; Highsmith & Eden, 1990). The transient electric birefringence measurements were made as in earlier studies (Highsmith & Eden, 1986). The rotational decay times were interpreted using the structural model of Iniesta et al. (1988).

RESULTS AND DISCUSSION

At 0 °C, ATP was added to a S1 solution, and the sample was rapidly transferred into the instrument cell which had been precooled to 3 °C. The temperature stabilized, and data collection was begun within 20 min after the ATP addition. A decay time for rotational Brownian motion, τ, with a standard deviation of less than 0.5%, could be obtained from

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[1] Abbreviations: S1, myosin subfragment 1; τ, time constant for the exponential decay of the electric field-free birefringence signal.

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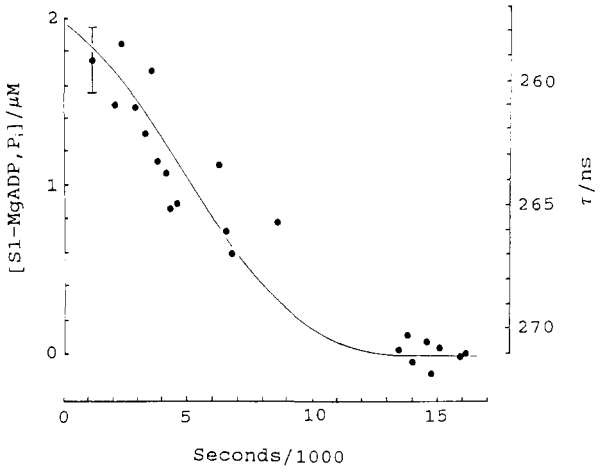

FIGURE 1: Ion exchange purified 2.50 μM S1 containing only alkali light chain 1 (Weeds & Taylor, 1976) was cooled to 0 °C in 10 mM Tris-OAc, 0.30 mM $MgCl_2$, pH 7.0 (at 4 °C). Stock ATP in the same buffer was added to obtain 2.0 μM S1 and 197 μM ATP. Measurements of rotational Brownian motion were made after ATP addition at the times shown. The mean rotational decay time, τ, was determined from a single-exponential fit to the birefringence signal obtained after removing a 6-μs 850-V electric field pulse across the 0.28-cm sample cell (Highsmith & Eden, 1986). The solid line is a calculation of the concentration of steady-state intermediate assuming Michaelis–Menten kinetics and using a rate of 0.014 s^{-1} [determined by a fluorescence method (Bachouchi et al., 1986) at 3 °C in the buffer used for the transient electric birefringence measurements], $K_m = 0.3 \times 10^{-7}$ M (Lymn & Taylor, 1970), and $K_i = 3 \times 10^{-6}$ M (Highsmith, 1976) for competitive ADP binding. The [S1-MgADP,P_i] were calculated assuming S1-MgADP,P_i is the exclusive steady-state intermediate, which is unlikely (Bagshaw & Trentham, 1974). The effects of the presence of other intermediates on the quantitative interpretation of the data are discussed in the text.

averaging 3 min of collected data. Measurements were made at intervals over the 4-h period required to convert the ATP entirely to ADP under these conditions. By making comparisons of S1-MgADP,P_i to S1-MgADP using the same preparation, the potential systematic errors due to variations in protein, ligand, or buffer preparations or concentrations were eliminated. The measured rotational relaxation time increases from $\tau = 259 \pm 1.5$ ns at early times to $\tau = 271 \pm 1.3$ ns at late times, corresponding to an increase in the hydrodynamic size of S1 as it changes from predominately S1-MgADP,P_i to S1-MgADP (Figure 1). The concentration of S1-MgADP,P_i, estimated from the measured rate of ATP hydrolysis under these conditions, follows a similar time course (solid line in Figure 1), which strongly supports the conclusion that the observed change in hydrodynamic size of S1 is ATP-dependent and corresponds to the S1-MgADP,P_i to S1-MgADP transition. The rotational decay time observed for S1-MgADP (271 ns at 3 °C) is consistent with previous measurements (Highsmith & Eden, 1986; Papp et al., 1992).

A change in hydrodynamic size can be due to a change in shape, flexibility, or state of aggregation. Transient electric birefringence and fluorescence anisotropy measurements indicate that aggregation is not significant under these conditions (Highsmith & Eden, 1986; Papp et al., 1992). Increased flexibility seems unlikely in light of the increase in fluorescence anisotropy and phosphorescence lifetime observed for S1-MgADP,P_i, which suggest a more rigidified internal environment in comparison to S1-MgADP (Kishi & Noda, 1983; Papp et al., 1992). An ATP-induced change in the shape of S1 that makes S1-MgADP,P_i more compact and therefore hydrodynamically smaller is the most likely explanation of the observed changes in the rate of rotational Brownian motion. This conclusion is consistent with the observation that S1-MgADP,P_i has a smaller radius of gyration than S1-ADP for chicken skeletal muscle myosin S1 with all light chains present (Wakabayashi et al., 1992). Rabbit and chicken skeletal muscle myosins are likely to function similarly, and the combined results suggest that light chain two does not have a significant effect on the S1 structural changes caused by MgATP. A rearrangement of S1 subdomains with little change in secondary structure is consistent with the results presented here and with the very small effect that ATP has on the circular dichroic spectrum of S1 (Johnson et al., 1991) or of heavy meromyosin (Cassim & Lin, 1975). Similar decreases in the hydrodynamic size due to ligand binding were shown originally for S1-MgADP binding to orthovandate to form S1-MgADP,V_i (Aguirre et al., 1989). These results have been confirmed (Highsmith & Eden, 1990; Cheung et al., 1991; Wakabayashi et al., 1992). In the case of S1-MgADP,V_i, the nucleotide is trapped, and it is unlikely that the protein has become more flexible (Goodno & Taylor, 1982). We have interpreted our results assuming that S1-MgADP,P_i and S1-MgADP are different in shape and not in degree of flexibility or aggregation.

Hydrodynamic data require a model for structural interpretation. We have used a bead model (Figure 2), in which S1 is represented as a string of four touching spherical beads of equal radii and the angle that is made by the lines that go through the centers of the first two beads and through the centers of the second, third, and fourth beads is varied in order to change its hydrodynamic size (Iniesta et al., 1988). This simple static model was chosen because it can be developed as a dynamic model that includes electric dipole moments on the beads and a restoring force at the bend point (Belmonte et al., 1991). An angle of 20° was used for the structure of S1-MgADP because the model then approximates to a reasonable degree the structure of S1 as it appears in electron micrographs of free S1 (Sutoh et al., 1989) or of S1 crystals (Winkelmann et al., 1991). The angle was then increased to obtain a decrease in rotational decay time that matches the one measured for S1-MgADP,P_i. The results obtained using this model and theory (Iniesta et al., 1988) indicate the angle decreases from 38° for S1-MgADP,P_i to 20° for S1-MgADP. For a S1 that is 20 nm long (Highsmith & Eden, 1986; Elliot & Offer, 1978), this transition displaces a point at the end of the longer assembly of beads by 3.9 nm (Figure 2A). These values obtained for the angles and the relative displacement due to the ligand-induced conformational change are model-dependent. Somewhat different values would probably be obtained if more realistic models of S1 structure were used, but the values obtained using the simple model in Figure 2 indicate that a substantial displacement of one end of S1 occurs when S1-MgADP,P_i is formed. The ATP hydrolysis-driven displacement of the thin filament by actin-bound cross-bridges in muscle has been estimated to be in the range of 5–12 nm (Huxley & Simmons, 1973; Huxley & Kress, 1985; Uyeda et al., 1991). If the ligand-induced shape change that is indicated by the measurements and analysis presented here occurs in muscle when myosin is bound to actin, it could account for a large portion of the ATP hydrolysis-driven filament sliding displacement with no reorientation of S1 at the acto-S1 interface (Figure 2B).

This value for the displacement caused by an ATP-induced S1 shape change is actually a lower estimate. The interpretation of the data in Figure 1 is based on the assumption that the only steady-state intermediate is S1-MgADP,P_i. However, at 5 °C the nucleotides bound in the active sites of the steady-

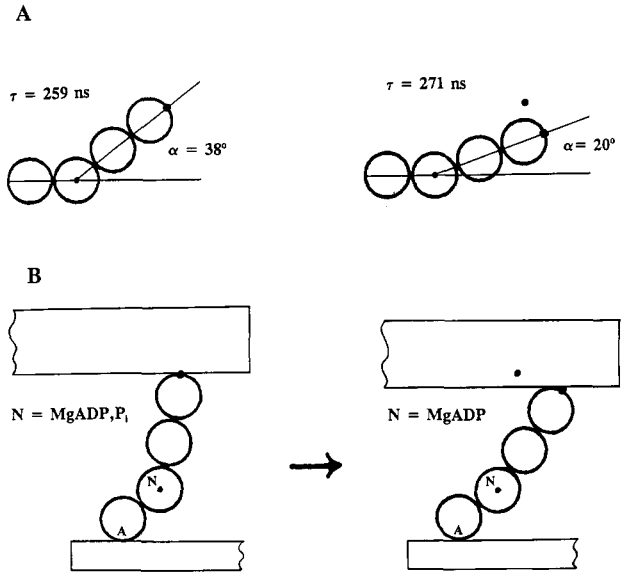

FIGURE 2: (A) A bead model and theory of the Brownian dynamics of bent rigid molecules in solution (Iniesta et al., 1988) was used to estimate the decrease in bend angle required to increase the mean rotational decay time from 259 ± 1.5 to 271 ± 1.3 ns. The angle for the hydrodynamically larger shape was chosen as 20° to obtain an S1 that resembles the current renderings of its shape (Sutoh et al., 1989; Winkelmann et al., 1991; Wakabayashi et al., 1992) in the absence of ATP. An angle of at least 38° was required for a structure with a decay time of 259 ns (see text). In the figure on the left side, there is a dot at the end of the long segment. In the figure on the right side, the dot above the bead model indicates how far the segment moved. (B) The bead model structures of S1-MgADP,P_i and S1-MgADP are depicted two dimensionally as they might exist as cross-bridges positioned between thick and thin filaments in muscle which are generating force to cause sliding. The locations of the ATP and actin binding sites on S1, shown as "A" and "N", respectively, are based on electron micrographs (Sutoh et al., 1989) and the interpretation of fluorescence resonance energy transfer measurements (Kasprzak et al., 1989). No rotation of S1 or actin occurs at the actin binding site. There is free rotation at the connection of the S1 to the thick filament (Mendelson et al., 1973). The S1 structural change is identical to the one shown in A above. The S1 intramolecular "rotation" elongates S1 and slides the thin filament. If the shorter segment of S1 is bound to actin, the end of the longer segment is displaced by at least 3.9 nm, when phosphate is released during the acto-S1-MgADP,P_i to acto-S1-MgADP transition. Reorientation of S1 and or actin at the actin binding site, if it occurs, could increase the displacement. The "dot" in the figure on the right indicates the position that the end of the long segment of the S1 model had in the figure on the left.

state intermediate species are distributed 55–64% ADP,P_i, 5–6% ATP, and 30–40% ADP (Bagshaw et al., 1974). The distribution will be somewhat different for our conditions, but we will use the published values for this discussion. The structure of the steady-state S1-MgADP is unknown. If the steady-state S1-MgADP is the same shape as the equilibrium S1-MgADP, the rotational times measured at early times are for a mixture that is about 65% hydrodynamically smaller S1 and 35% hydrodynamically larger S1, rather than 100% smaller as assumed in the above analysis of the data in Figure 1. If this is the case, the decay time is 253 ns for the hydrodynamically smaller S1, and the structural models in Figure 2A require a change in angle from 45° for S1-MgADP,P_i to 20° for S1-MgADP. This increases the displacement of the end of the longer segment of S1 from the 3.9 nm estimated above to 5.4 nm. If this four-bead model is used to calculate the changes in radius of gyration from the changes in bending angle determined from τ, one obtains 2.1% and 3.4% for the 38° and 45° to 20° changes, respectively. These values are in reasonable agreement with the 3.7% change that was determined by small-angle X-ray scattering from S1-MgADP,P_i and S1-MgADP (Wakabayashi et al., 1992) and gives us confidence that the model, although simple, is quantitatively useful.

The idea that free S1 interacting with ATP would produce a more compact S1 structure that would do work by returning to its longer conformation when it binds actin is physically, mechanically, and energetically reasonable. ATP appears to bind to S1 in a two-step mechanism (Johnson & Taylor, 1978) which provides increasing protection from the solvent (Ando & Duke, 1983), suggesting that the binding site is a crevice. Nucleotides can be trapped in the active site by a variety of methods (Wells & Yount, 1982), which is consistent with a crevice that can close. The rotation of subdomains to close an active site onto its substrate and produce a more compact protein have been shown for other proteins (Lesk & Chothia, 1984; Sharff et al., 1992). If S1 does become more compact as it binds to MgATP, it is an example of what has been called conformational coupling (Boyer et al., 1975). The force-generating step or "power stroke" in muscle or motility assays would be the reverse of this ligand-induced conformational change, which occurs as P_i and MgADP are released from the actin-bound S1.

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